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Hypoglycemic Activity Of Ethanolic Extract Of Aphyllorchis montana Induced By Streptozocin In Rats

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Abstract: The present investigation was carried out to study the hypoglycemic effects of the methanol and acetone (70:30) extract of *Aphyllorchis montana*, in normal and streptozocin induced diabetic model. *Aphyllorchis montana* are reported to have medicinal values including hypoglycemic properties. Decreased blood glucose level of the test animals shows that the extract exhibit significant hypoglycemic activity when compared to diabetic control group, effect of various doses (100, 200mg/kg, p.o) extract was studied on streptozotocin induced both diabetic and non-diabetic rats. The results also indicated the dose dependent effect. The hypoglycemic activity produced by the extract may be due to increased uptake of glucose at the tissue level or by an increase in pancreatic beta cell function or due to inhibition of intestinal absorption of glucose. The present study supports the use of this herbal drug as hypoglycemic The reduction in the glucose level in induced diabetic rats proved that *Aphyllorchis montana* having the wide hypoglycemic activity.
Key words: *Aphyllorchis montana*, hypoglycemic, streptozocin

INTRODUCTION

Diabetics have significantly accelerated levels of oxidative stress and this contributes massively to most neurological, cardiovascular, retinal, renal diabetic complications [(1)]. Diabetes mellitus is a metabolic disorder characterized by fasting hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and action [(2)]. Experimentally, streptozotocin (STZ) or alloxan are used to induce diabetes in rodents. STZ is effective in triggering islet cell death by acute oxidative stress. STZ-induced diabetic rats are one of the animal models of insulin dependent diabetes mellitus characterized by high fasting blood glucose levels and drastic reduction in plasma insulin concentration [(3)]. Although different types of oral hypoglycemic agents are available along with insulin for the management of diabetes mellitus, there is a growing interest in herbal remedies due to the side effects associated with these therapeutic agents [(4)]. Thus plants have played a major role in the discovery of new therapeutic agents. The present study was undertaken to investigate the anti-hyperglycemic effect of the methanol and acetone extract of *Aphyllorchis montana* on the diabetes induced by a multiple dose of STZ in diabetic rats.

MATERIAL AND METHODS

Plant material

The leaves of *Aphyllorchis montana* used in the present study was collected from the natural habitat in and around Chennai, Tamilnadu and the plant material was authentified by Dr.P.Jayaraman Ph.D., PlantAnatomy Research Centre (PARC),Tambaram. Voucher number is PARC/2010/803.

Animals

Wistar Albino rats of either sex, weighing 150–200 g, were used in the study. They were kept in standard laboratory conditions under natural light and dark cycle, and are housed at ambient temperature (22±1°C), relative humidity (55±5%). Animals had access to standard pellet diet and water given *ad libitum*. The experimental animals had approved by our institutional ethical committee following the guide lines of CPCSEA. The proposal number submitted CPCSEA was IAEC/2011

Induction of diabetes

Streptozotocin-induced diabetes

Streptozocin was obtained from Himedia Laboratories, Mumbai. All other chemicals used for this study were of analytical grade. Streptozotocin (55 mg/kg) was dissolved in 0.1M citrate buffer (pH 4.5). Six rats per group were administered by subcutaneous injection. After 48 h, fasting blood glucose levels as well as glycosuria were assessed to confirm the diabetic state. Only rats with a fasting blood glucose level of at least 250 mg/dL and positive urine glucose were considered diabetic and were used in the experiment.

Experimental Design

Male Wistar albino rats weighing 150–200 g (90 to 110 days old) were used. The animals were randomly divided into five groups of six animals each.

Group 1: Normal control (non-diabetic, untreated) rats.
Group 2: Diabetic control (diabetic, untreated) rats.
Group 3: Diabetic test rats given *Aphyllorchis montana* extracts at the dose of 100 mg/kg.
Group 4: Diabetic test rats given *Aphyllorchis montana* extracts at the dose of 200 mg/kg.
Treatment of experimental animals with plant extracts was initiated 2 days post streptozotocin injection and was carried out once daily, by orally, for 14 days. Food and water were made freely available.

Measurement of body weight gain, food, water intake and blood glucose

Body weight gain, food and water intakes were monitored daily during the 14 days experimental period. Blood samples for glucose determination were obtained from the tail tip of 12 h fasted rats on day 0 (before streptozotocin administration), days 2 (48 h post streptozotocin injection), 5, 8, 11 and 14th day of the experiments. Blood glucose level was determined using a glucometer (Accu-Check, Roche). Urine glucose was also assessed in fresh urine using glucose indicator sticks (Boerhinger Mannheim, Germany) before and 48 h after streptozotocin administration, for the confirmation of the diabetic state of animals.

Statistical Analysis

Mean values were obtained by one-way analysis of variance (ANOVA) followed by Dunnet's't' test, using the computer software, Graph pad Prism 5. The significance of difference between and within various groups was determined. The results are expressed as mean ± S.E.M. Values of $p < 0.05$ were taken to imply as statistically significant.

RESULTS AND DISCUSSION

The effects of the *Aphyllorchis montana* extract on the body weight of diabetic rats are shown in Table 1.

During the 2 weeks of observation of the extract treated diabetic rats at doses of 200 mg/kg, there were very significant ($p < 0.01$) weight gains relative to day 2. The diabetic rats treated with three units of insulin also showed a very significant ($p < 0.01$) weight increase in the body compared to untreated diabetic rats.

Table 2 shows the effects of the extracts on food and fluid intakes by diabetic rats. When compared to the untreated diabetic rats, untreated diabetic rats had severe polyphagia and polydipsia at the end of the second week of the experiment with respective increase in food and fluid intakes. However, in the presence of *Aphyllorchis montana* extracts extract (100mg/kg and

200 mg/kg), food intake was reduced when compared with diabetic control rats but it's not statistically significant ($p>0.05$). Fluid intakes showed decrease in *Aphyllorchis montana* extracts treated diabetic rats at doses of both 100 mg/kg and 200 mg/kg when compared with diabetic control rats. Diabetic rats treated with three units of insulin also showed a non-significantly lower water intake ($p>0.05$).

Table 3

Following a 48 h post streptozotocin injection, all diabetic rats exhibited hyperglycemia, which the glycemic level of 200 mg/kg *Aphyllorchis montana* extract treated diabetic rats dropped significantly.

In diabetes, oxidative stress is due to both an increased production of plasma free radical concentration and a sharp reduction of antioxidant defenses. GSH, being the most important bio-molecule against chemically induced toxicity can participate in the elimination of reactive intermediates by reduction of hydro peroxides in the presence of Glutathione peroxidase. Glutathione (GSH) also functions as free radical scavenger and in the repair of free radical caused biological damage[(5)]. The important mechanism implicated in the diabetic action of STZ is by increased generation of oxygen free radicals, which causes a decrease in plasma GSH concentration, and plasma GSH/GSSG ratio[(6)]. Our results suggest that the methanol and acetone *Aphyllorchis montana* extracts have dose-dependent hypoglycemic activities on streptozotocin-induced diabetes[(7)]. The metabolic disturbances were corrected after the plant extracts were administered for 2 weeks, as shown by the normalization of fasting blood glucose levels, reduction in polyphagia and polydipsia and weight gain by diabetic-treated rats but reduction in polyphagia and polydipsia are not statistically significant[(8)]. The mechanisms by which streptozotocin brings about its diabetic state include selective destruction of pancreatic insulin secreting beta cells[(9)], which make cells less active and lead to poor glucose utilization by tissues. *Aphyllorchis montana* significantly reduced the high fasting glucose levels [(10)] in streptozotocin-induced diabetic rats. This suggests that the extracts may possess insulin like effect on peripheral tissues by either promoting glucose uptake and metabolism[(11)],by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues by the stimulation of a regeneration process and revitalization of the remaining beta cells[(12)]**.**

Table1: Effects of *Aphyllorchis montana* extracts on the body weight

S.no	Groups	Body weight on administration	
		2days after injection	14days after injection
1	Normal control	204.0 ± 3.821*	219.5±3.227**
2	Diabetic control	192.2 ± 3.541	168.5±3.227
3	Test-1(*Aphyllorchis montana* 100mg/kg)	196.6 ± 1.631*	176.0±4.708*
4	Test-1(*Aphyllorchis montana* 200mg/kg)	197.1 ± 1.631*	182.5±5.204**

Results are expressed as mean ± SEM, n=6

Table2: Food and fluid intakes of rats treated with *Aphyllorchis montana* extracts

S.no	Groups	Food intake (mL/rat/week)		Fluid intake (mL/rat/week)	
		Week 1	Week 2	Week 3	Week 4
1	Normal control	60.50 ± 1.683	62.00±1.291	23.50±3.27*	20.25±1.493
2	Diabetic control	67.50±2.630	69.50±2.533	22.50±3.22*	23.25±1.493
3	Test-1(*Aphyllorchis montana* 100mg/kg)	59.00±1.683	54.50±1.041	32.25±2.175	42.25±3.326
4	Test-1(*Aphyllorchis montana* 200mg/kg)	58.00±1.472	50.25±1.250	38.00±2.483	39.50±1.708

Results are expressed as mean ± SEM, n=6

Table 3: Blood glucose level (mg/dL) of rats 2 days post STZ administered and after 14 days of treatment with plant extracts.

S.no	Groups	Glycemia (mg/dL)		
		Before STZ	2 Days after STZ	After 14 days treatment
1	Normal control	105.0 ±6.455	110.0±4.564**	105.5±2.723**
2	Diabetic control	108.8±4.270	387.8±7.375	373.5±6.886
3	Test-1(*Aphyllorchis montana* 100mg/kg)	111.3±4.270	369.8±4.090	120.3 ±4.442**
4	Test-1(*Aphyllorchis montana* 200mg/kg)	103.8±8.985	362.3±5.977*	116.8±4.854**

Results are expressed as mean ± SEM, n=6

CONCLUSION

In conclusion the present investigation showed that *Aphyllorchis montana* extract possess hypoglycemic activity. *Aphyllorchis montana* extract showed the effect due to enhancing effect on cellular antioxidant defenses to protect against oxidative damage.

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